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THE REDUCTION DIVISIONS IN THE POLLEN MOTHER CELLS OF OENOTHERA FRANCISCANA

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Introduction

Investigation of the Oenotheras has reached such a stage that it is essential, if any progress is to be made in the study of the group, to obtain species concerning the purity of which there can be no question. Of all the species so far studied, *Oe. franciscana* seems to give the most promise of being undoubtedly pure. This is a far western species, which was first described by H. H. Bartlett in 1914. His description is based on material grown at Washington for the first time in 1910, from seed collected at Carmel Beach, California, in 1905. Seed from this strain was sent to Dr. B. M. Davis in Philadelphia, and grown by him under the name of *Oe. franciscana* B., which race he has carried on in selfed line since 1913. This strain has proved to be very stable. It has never thrown any mutants, has shown a high percentage of pollen and seed fertility, and in every respect acts like a well established pure race. It thus falls into a group with *Oe. biennis* L. and *Oe. grandiflora* Ait., which have also been shown to be very constant. The cytology of *Oe. grandiflora* has been worked out by Davis (1909) and has proved exceedingly interesting as revealing a very regular nuclear behavior during the reduction process, with a definite pairing of homologous chromosomes at diakinesis and the formation of a clear equatorial plate at the heterotypic metaphase. This uniformity in development is quite in contrast with what has been found to be the case in those Oenotheras which are less stable; and it therefore becomes of interest to examine the spore mother cells of the other stable species to determine whether they also may show the same regularity of development displayed by *grandiflora*.

Material

Collections were made by the writer during the summers of 1919 and 1920 in the gardens of Dr. B. M. Davis. A variety of fixing fluids were used. Of these, one of Allen's modifications of Bouin's solution (Bouin A) gave the best results, although strong Flemming with urea and maltose added (S.F.U.M.) yielded some fairly satisfactory fixations.

Description

An attempt was made to study carefully the telophase stages of the last archesporial mitoses, with a view to determining whether a definite splitting of the chromosomes into two parallel threads takes place, as described by Digby (1919), or whether the events conform rather to accounts such as those given by Sharp for Vicia (1913) and Tradescantia (1920). It was found impossible, however, to get very definite results, as the nuclei at this stage were too small, and the chromosomes too tightly packed to allow of close observation. The following account, therefore, begins with the return of the nucleus to a resting condition after the last archesporial division.

The cells of the archesporium pass through a fairly long-continued resting stage previous to entering upon the maturation prophase. They lie usually in one or two rows and fit tightly together with no spaces between them or between the archesporium and the well-differentiated tapetum. The nucleus is comparatively large (10–11 μ), very nearly spherical, and possesses a prominent nuclear membrane (Pl. XXV, figs. 1, 2). Within it may be found one or more nucleoli. Ordinarily but one of these attains to any size. It is spherical, and lies toward the center of the nucleus. Within it may often be seen one or more hyaline circular areas, resembling vacuoles. These may contain, or occasionally give place to, angular, crystal-like bodies. The other nucleoli when present are usually smaller and are stationed here and there throughout the nucleus. All of these bodies are in intimate contact with the linin network.

Scattered evenly throughout the nucleus is a reticulum of very delicate threads. Some of the threads are very thin and free from granules. Others are dotted over more or less unevenly with chromatin particles. Wherever threads join, chromatin accumulations varying in size from tiny dots to fairly large masses are to be found. These may be irregular and shapeless, or rounded and indistinguishable, perhaps not different, from small nucleoli. In well fixed material the network closely envelops the nucleolus, to which it is connected in various places. There is, therefore, no space around the nucleolus. Wherever the fixation is faulty, however, a clear space is observed, due to shrinkage of the reticulum. During the resting stage there is little if any indication of parallelism of the threads. For the most part they are single and are uniformly distributed throughout the nucleus.

The resting condition lasts for quite a time, long enough for the anther practically to double in length. Throughout this period all trace of the individual chromosomes is lost—they have been resolved completely into a network of threads.

Although there is little or no sign of parallelism of threads during the resting stage, there begin to appear occasional instances of such an approximation as the nucleus begins to draw near to the time when it will enter upon the heterotypic prophase (fig. 3). By no means all the threads become thus arranged. Most of them remain single. Moreover, the parallel threads do not come together very closely, and there is not much assurance at this stage that a fusion of the approximating threads will result. The condition is sufficiently different, however, from the typical resting condition to be noticeable and to indicate that the nucleus is approaching prophase.

The Heterotypic Prophase

The series of events which follows may be briefly defined as a process by which a small-meshed reticulum composed of many thin, often more or less unevenly granular threads becomes transformed into a large-meshed reticulum composed of fairly thick and uniform threads—a network of such large mesh, and with the threads joined to each other in so few places, that it can perhaps be better described as a spireme. The problem before us for the moment is to determine by what means this change, with the consequent reduction in the number of threads, is brought about.

The researches of Grégoire and Allen and their followers, on the one hand, and of Farmer, Digby, Mottier, and others on the other, have been in agreement as indicating that a most important element in this process is a side-by-side approximation and fusion of threads in early prophase, thus reducing the number of threads to approximately one half. For every thread there is a counterpart in the reticulum, which is so placed that the two can approach each other and fuse. The interpretations placed upon this process, however, by the two schools have been different. The former group (the parasynaptists) consider that the threads which enter into the fusion are homologous parts of two spiremes, one of which traces its ancestry back to the egg, the other to the sperm. The threads represent whole chromosomes and their fusion results in a bivalent spireme. A typical telosynaptic interpretation has recently been set forth very clearly by Miss Digby in her paper on Osmunda (1919). According to this view, the individual threads are half-chromosomes which form on "association" a univalent filament, the chromosomes descended from both sperm and egg forming a part of one spireme. Miss Digby has described in the last telophase before the heterotypic mitosis a longitudinal fission of the chromosomes into two parallel threads, which are later obscured in the resting stage. The parallelism in prophase she considers as due merely to the return of these two split parts together. According to this view, the fission in telophase is preliminary to the separation of chromosomes during anaphase of the homoeotypic mitosis. According to the Gregoirean interpretation, the two parts which fuse in prophase will separate during anaphase of the heterotypic mitosis.

While in general the method of development in *Oenothera franciscana* is telosynaptic, we shall nevertheless see that there is no evidence of any very general parallelism and side-by-side fusion of threads in early prophase such as has been observed in Osmunda. It is true that some parallelism is apparent at the very beginning of prophase, as I have already pointed out, and without doubt a certain part of the condensation of the network is effected through the agency of parallel fusions, but by far the greatest amount of it is brought about in other ways.

As the nucleus advances into prophase, the amount of parallelism does not increase materially, but the reticulum loses the even and uniform appearance that has been so characteristic of it (fig. 4). The meshes begin to show a marked difference in size, the threads are no longer so evenly distributed, and are becoming quite unequal in diameter. The difference in size of the meshes seems to be due mainly to the fact that some of the threads are contracting and becoming thereby shorter and thicker. This process serves to bring threads which have been lying far apart nearer together, so that they occasionally parallel one another quite closely. It also helps to bring about a variation in the diameter of the threads. Those which contract will become thicker thereby. Furthermore, contraction on the part of some threads will result in the stretching and attenuation of others. Another process seems to be going on at the same time, however, which is doing much to make some threads thicker than others. There are indications of a flow of material from one thread to another, or from one part of a thread to another part, resulting in a marked thickening of some parts and the absorption and disappearance of others. One may observe everywhere threads whose contents seem to have passed to adjacent knots or threads, causing these to enlarge, while the former have themselves become very thin and are either on the point of breaking or have already broken (figs. 4–7). It is evident that certain threads are being sacrificed, and that the material of which they are composed is being transferred to the threads which are to survive, or is being temporarily stored in the "knots," formed where the threads join. A process of this kind, involving a certain amount of contraction, will necessarily result in occasional parallelisms. They are not sufficiently numerous in this material, however, to warrant the belief that they are of any far-reaching significance. They are what might be termed "chance" parallelisms.

Synizesis. The condition of synizesis is apparently brought about by the continued contraction of the threads of the network. There is so much pull exercised upon the peripheral threads because of this shrinkage that they become torn away from the nuclear membrane on one side, and the contracting mass of threads shrinks into a bundle against the opposite side of the nucleus. Either before this breaking away occurs or while the bundle is being formed, the nucleolus leaves its central position and comes to lie against the membrane in the midst of the reticulum (figs. 4, 5, 8, 9). Here it becomes somewhat flattened and cushion-shaped.

The position of the synizetic knot is of interest. One cannot help being struck by the fact that during synizesis the threadwork lies, in the vast majority of cases, on that side of the nucleus which is nearest the periphery of the cell. Just what significance should be attached to this fact I am not prepared to say. The question naturally arises, however, as to whether such a position might not be due to strong exosmotic currents which would pull the reticulum toward that side of the nucleus first affected by the fixing fluid. If it be true that the position of the knot is due to the action of the fixing fluid, one might perhaps be justified in harboring slight suspicions as to the cause of the shrinkage itself.

In early synizesis the whole reticulum is so massed together and the individual threads are so fine that it is almost impossible to distinguish anything in detail. The only way really to observe what is taking place is to study thin tangential sections of the knot. We are in this way enabled to see that the same process which was going on just previous to synizesis, by which certain threads were being absorbed while others were becoming enlarged, is still in progress. The network is at first much like that which entered synizesis, except that the meshes have become smaller by contraction, and the whole has thus become much more compact (figs. 5–7). The threads, however, rapidly become less and less uniform in appearance, as certain of them give up their contents to others (fig. 6). As the result of this process, some of the threads begin to stand out more and more prominently, so that, as later and later stages are reached, it becomes apparent to one examining the knot as a whole that it is reticulate in nature. One can distinguish threads running here and there throughout the densely packed mass. Tangential sections of late stages show that the finer threads are fast disappearing and that those which remain are becoming not only more prominent, but on the whole more uniform in diameter and less granular (figs. 8–10).

Certain of the threads in the center of the reticulum, and often in close juxtaposition with the nucleolus, may in time become especially prominent (Pl. XXV, figs. 9–12; Pl. XXVI, fig. 13). They swell greatly and evidently come to contain a great deal of chromatin material. A whole section of the network may become thus involved, with the result that a large, more or less shapeless body is formed in the middle of the reticulum. The nearness of this mass to the nucleolus suggests that the latter may be emptying itself of its chromatin contents, the rate of emptying being greater than the rate at which it can flow out into the various parts of the reticulum. These aggregations disappear more or less completely by the beginning of the open spireme stage (fig. 14).

During late synizesis the spireme becomes continually clearer and more uniform, as the threads which are not to survive disappear and their material, and that from the nucleolus, becomes more evenly spread over the thread system (Pl. XXV, figs. 8–10). While the surviving threads are much thicker than those of the pre-synizetic reticulum, they do not continue to thicken indefinitely. Instead, they lengthen considerably to make room for the additional chromatin material. This lengthening process may very well be the cause of the unfolding of the tangled knot. A reticulum of such long threads cannot remain in such a small space unless the threads are very sharply and frequently bent and twisted. There is a tendency to resist this excess of bending, to straighten out, and hence the unfolding.

The nucleolus, which at the beginning of synizesis moved over against the nuclear membrane, becomes by mid-synizesis not only flattened but much spread out and quite often assumes the shape of a horseshoe or doughnut. That it is comparatively empty is shown by the staining and by the fact that an endonucleolus now stands out very clearly within it as a black-staining, spherical little body. This body makes its first appearance in early synizesis and remains a constant feature of the nucleolus until the time of the disappearance of the latter during diakinesis (fig. 11).

Synizesis is, then, the time during which transition is made from a thin-threaded reticulum with scattered chromatin particles to a thick-threaded spireme. This transition does not appear to be brought about by the parallel fusion of two separate thread systems, but by a process which consists largely of the contraction of certain threads and the absorption of their contents into the body of other threads, a process which involves only occasional parallelisms. It seems, also, that the total amount of chromatin on the threads is greatly augmented during this process, and it is not unlikely that the extra chromatin has been brought out of the nucleoli and distributed along the network.

The Open Spireme. The unfolding of the synizetic knot is gradual. It is accompanied by little further thickening of the spireme. The large irregular accumulations of chromatin which were formed in the center of the knot during synizesis are dwindling, though the threads at the center are usually found to be somewhat thicker than the rest of the network through the whole of the open spireme stage (Pl. XXV, fig. 12; Pl. XXVI, figs. 13–15).

Judging from the frequency of its appearance, the open spireme stage is one of fairly long duration. The threads are long and evenly distributed through the nucleus (fig. 14). They frequently show a distinct and regular beading. It is often possible to trace a single thread for a long distance as it winds and twists through the nucleus. Here and there it joins with other threads, the whole system constituting not a single coiled or twisted thread but a loose reticulum. Ordinarily it is not possible to locate any threads with free ends.

During the open spireme stage the threads are characteristically single and unsplit. Wherever they are prominently beaded, the beads are found to be arranged in a single row, stretching quite across the thread and showing no sign of division. Once in a while one may find in late synizesis a lingering trace of parallelism, but in the open spireme we rarely have anything to indicate that the thread might be by nature double. Occasionally two parts of the spireme may lie rather close together, and the parallelism may strike one at first, but it is quickly evident that this is merely the chance lying together of two whole threads which, when traced a little distance, are seen to have no immediate connection with one another. During this stage, also, the nucleolus begins to round off and leaves the nuclear membrane, where it has been lying through synizesis. It usually takes the stain very lightly so that the endonucleus shows clearly within. It is an interesting fact that the latter body seems quite often to be clearly connected with a part of the spireme (fig. 16).

During synizesis and the open spireme stage, the pollen mother cells, which during the resting period were packed closely together, begin to round off and to separate somewhat from each other, though they do not become entirely detached. The anther and the loculus in which they are placed become considerably enlarged during this time, but the cells themselves grow only slightly. The nuclei also do not grow appreciably, measurements during the open spireme and second contraction stages showing an average diameter of 12–13 μ, as compared with 10–11 μ in the previous resting phase.

Second Contraction. After a time the nucleus begins to pass out of the open spireme stage and to enter upon the stage known as "second contraction." The spireme, which has hitherto been rather evenly distributed throughout the nucleus, begins to thicken and shorten, the central portion condensing very rapidly, while the peripheral part becomes thrown into loops which radiate more or less clearly from the central region (fig. 15). The threads in the center of the nucleus become thicker and thicker, until they gradually lose their thread-like form and become great, irregular, swollen masses which, as they increase in size, come to lie against one another so closely that the individual parts become lost and it is usually impossible to tell whether they have amalgamated or not (figs. 16–18). This process results from the passage of material into the region from the rest of the spireme, a process which results also in a shortening of all the threads of the reticulum.

As condensation goes on and this central mass becomes larger and larger, all the threads of the reticulum shorten, and it becomes more clearly evident that the threads which pass out toward the periphery are arranged in loops (figs. 15–18). Even in early stages of the second contraction it can be seen that the threads are forming loops which run out to the periphery and which are attached to each other in places (fig. 15). At first they are so long that they must bend in various ways, but as the threads shorten, the loops become smaller and smaller, so that at last they can stretch their full length without touching the periphery. The nucleus then begins to present a very striking figure, the chromatin mass resembling a large, irregular central hub from which the loops radiate out like spokes in a wheel (fig. 19). As the loops become shorter, their two sides may begin to approximate until in some cases they actually lie against one another (figs. 19, 20). They do not twist around one another, however, to form the characteristic condition known as strepsinema. When the climax of condensation has been reached, the loops are sometimes so short that they are barely visible beyond the edge of the central mass (fig. 21). In practically no case, however, are they entirely lost sight of. Throughout the whole of this period at least some of the loops are in evidence, and it seems certain that they form the bivalent chromosomes which are soon to emerge from the contracted mass.

The nucleolus is usually not involved in this condensation. During the second contraction it ordinarily retreats again to the nuclear membrane, where it begins to flatten out once more. It still shows as clearly as ever the black-staining endonucleolus (figs. 18–20, 23).

It is important to notice that there is almost no indication of the occurrence of any kind of split in the spireme during this process of contraction. In only a very few instances have I found any trace of it, and these were in poorly fixed cells. It is possible that about or before this time the spireme begins to divide lengthwise in preparation for the homoeotypic division, and that this split occasionally shows. If the spireme were bivalent in nature, however, we ought to find at this stage, when the chromosomes are preparing to separate from each other, that the spireme is everywhere splitting into two threads. I have been unable to find evidence that such a splitting occurs. The thread is constantly single.

As the loops become shorter and thicker, the first indications of the approaching cross-segmentation of the spireme make their appearance in the form of constrictions (figs. 16, 19, 21). A constriction may be found at the distal end of the loop, in which case each side of the loop represents a chromosome. In some cases a single chromosome makes up the whole of the distal portion, its ends being found part way down the sides (fig. 19). Such a loop might be made up of two, or of more than two chromosomes. The distal chromosome might be attached at both ends to the same chromosome, the middle part of which is hidden in the central mass, or the ends might be in contact with different chromosomes. We shall see shortly that the latter condition is probably always found in the case of one of the loops.

That the chromosomes are placed end to end in the spireme which makes up these loops is indicated by the fact that the individual loops may be traced entirely through the second contraction stage without showing any longitudinal splitting, emerging at the end of the period, each as a pair of chromosomes which are attached to each other by their ends. There is little doubt in my mind but that the segmentation which appears in the loops as these are contracting represents the separation of univalent chromosomes and not of bivalent ones.

Diakinesis. Up to this point we have been dealing with the spireme as a whole. During the earlier stages of prophase the thread system has been too long and complexly woven, and in the second contraction the massing of the material in the center has rendered conditions too obscure, to permit a study of the individual chromosomes to be made. From now on, however, it becomes increasingly possible to make such a study, and to determine what relation the individual chromosomes bear to each other and whether or not this relation is uniform. The conditions that we find are very interesting.

When the second contraction knot begins finally to loosen, it does so in a definite way. It lengthens out, and from a more or less rounded, spherical mass assumes a roughly cylindrical form (figs. 22, 23). As it grows longer, the individual bivalent chromosomes which compose it begin to show more clearly (fig. 23), and, despite the fact that the figure is still obscurely massed in the center, their appearance suggests a definite arrangement. From one end of the figure it is common to find a single loop or ring protruding, and from the other end, two loops. Various others may be seen emerging from the sides of the central cylindrical portion. At this time the exact arrangement of the chromosomes is not clear, but the general cylindrical shape of the whole, and the uniform placing of such loops as can be seen clearly, indicate that they do have a definite positional relationship to each other.

As the figure opens out more and more and the nucleus passes into diakinesis, it becomes possible to make out the arrangement with greater clearness. The central part of the figure resolves itself into a closed circle, composed of four univalent chromosomes attached end to end (fig. 24). Linked to this circle at different places are rings, each of which is composed of two univalent chromosomes which are attached to each other at both ends. To certain of these rings there is linked in turn another ring made up in the same way. It is possible to count these rings in most cases, and we find that there are five of them present, which, together with the circle of four chromosomes, make a total of fourteen chromosomes, a circle of four and five pairs.

The chromosome pairs begin to separate from each other almost as soon as the second contraction knot begins to loosen, so that it has been impossible to find a case in which all the chromosomes were still occupying their original position with reference to one another, after the knot had completely loosened. Fortunately, however, the rings do not seem to separate in the same order at all times, so that it has been possible by studying many cases of linking to get quite a definite idea of the original arrangement; and in spite of the fact that I have found no case in which they have all remained together long enough to be clearly observed, I have become quite convinced that there is a definite arrangement of the chromosomes which is rarely if ever disturbed. In the first place, it is certain that the closed circle of four chromosomes is practically always present. I have found no case in which it was certainly lacking, but every good nucleus in diakinesis has displayed it beyond question; so that it seems perfectly clear that this grouping together of four chromosomes is a natural and regular proceeding. In the second place, a study of the various ways in which the bivalent rings are found linked to this circle and to each other in early diakinesis has made it seem very probable that here also we are dealing with a very definite arrangement. No more than three rings have ever been found attached to the circle of four. No more than two of these rings have been seen in a nucleus with other rings linked to them, and there have been no instances observed in which more than two rings were certainly linked together (Pl. XXVI, fig. 24; Pl. XXVII, figs. 25–27). The presence of three rings linked to the circle of four has been quite often seen, however, and the two two-linked chains are frequently in evidence. These facts taken together would seem to indicate that before the bivalent chromosomes begin to separate, there are three pairs or rings which are linked to the circle of four, and that two of these rings have linked to them in turn each another ring, making five rings in all. Judging from the frequency with which the various combinations occur, it is probable that the ring which has no other ring attached to it is most often the first one to break loose from the combination. Occasionally, however, one of the other rings will come off first.

Such a definite linking of chromosomes is especially interesting for two reasons. First, it suggests very strongly that at earlier stages also the chromosomes may have a definite position in the spireme, so that when the latter condenses to form the chromosomes, they will always find themselves linked in the same way. Such a uniformity of linking as is seen in early diakinesis can be accounted for in only one way, as I see it, and that is by assuming that when the chromosomes are yet in the spireme condition they always occupy the same relative positions, and that the individual threads of the spireme are also definitely placed, connecting with each other at the same points and interweaving in the same manner in all nuclei. Furthermore, it seems to me likely that this definite arrangement does not begin with the spireme stage, but goes back through synizesis into the resting condition. It may be that the chromosomes in somatic cells also are always arranged in the same way.

In the second place, this uniform arrangement is interesting because it furnishes a very good bit of evidence for the telosynaptic arrangement of the chromosomes and for the univalence of the spireme thread. If the homologous chromosomes were arranged side by side in the spireme, it is difficult to see how linking could occur. It could easily be brought about, however, if the chromosomes were to represent segments of the whole spireme placed end to end. The arrangement would then be brought about naturally in condensation of the spireme because of the original position of the threads which later become the chromosomes.

The bivalent rings usually separate from each other and from the circle of four after a time (fig. 28). The circle of four, however, does not break up in diakinesis, but remains until the disappearance of the nuclear membrane, and even later, being often seen during the formation of the equatorial plate and even occasionally until metaphase (fig. 29). In some cases one or more of the rings remain linked to the circle of four until the end of diakinesis, but more usually we find in late diakinesis that they have all separated, so that we have the circle of four, and five pairs of chromosomes scattered about in the nucleus. One of the most interesting features of this stage is its remarkable clarity. The chromosomes are clearly distinct from one another, often attached together only by narrow threads so that they stand quite apart. The presence of such a circle of four chromosomes has been observed before. Miss Digby (1912) has described this condition in one of the Primula hybrids. Her figures undoubtedly testify to its existence in her material, but it evidently does not show as clearly as it does in *Oenothera franciscana.*

During diakinesis the nucleolus is plastered against the nuclear membrane and is fast disappearing. At times it appears vacuolated, at others it is evenly translucent and apparently empty except for the black-staining endonucleolus which is often still prominent. The nucleolus seems to be melting. The edges are becoming hazy and ill-defined, and the structure looks as though it were being dissolved away. I have seen the endonucleolus in a few cases apparently in the act of being liberated, through the melting away of the nucleolus, and it is very probable that this little body maintains its identity for some time after the nucleolus has disappeared.

Metaphase

It is not until the nuclear membrane is on the point of breaking down that signs of spindle formation become evident. The first indication of this formation is a sudden increase in density of the cytoplasm close to the nuclear membrane. At first no fibrils are to be seen. These do not appear until the nuclear membrane begins to dissolve. Then they suddenly make their appearance on all sides, and, as the membrane melts, penetrate into the nuclear region. Within a short time, a very prominent multipolar spindle is formed, with several thick clusters of prominent fibrils. As these pass into the nucleus, and in and around the chromosomes, those of each cluster anastomose in various ways with fibrils from other bundles (fig. 29).

While the spindle is being formed and the nuclear membrane is disappearing, the chromosomes rapidly shrink, until they are less than half the size which they have presented during diakinesis. They have probably had a somewhat spongy texture, and are now being greatly compacted. During the whole of the period when the spindle is still multipolar, the circle of four remains as a rule unbroken, and even shows frequently one or more rings still linked to it. During this stage also a small but prominent black-staining little body is usually in evidence, which can hardly be other than the endonucleolus, which appears to outlast the nucleolus in most instances. There is no evidence, however, that this is a permanent structure which is being carried over into the daughter nuclei. It soon after disappears and probably suffers a dilatory but like fate with the nucleolus.

The chromosome pairs are drawn to the center of the cell as the spindle becomes bipolar, and soon become arranged in a definite plate. The circle of four soon breaks into two pairs, so that there come to be seven pairs of chromosomes lying side by side in the equatorial region. The condition at this time is generally one of great regularity. Most of the pairs are still in the form of rings, and one could not imagine a more typical picture of metaphase. The appearance is especially striking after the univalent chromosomes begin to be pulled apart, and before they have actually separated (fig. 30).

Anaphase and Telophase

The spindle fibers usually attach themselves to the middle of the chromosomes, which are V-shaped as they pass to the poles (figs. 30, 31). Occasionally, however, chromosomes are fastened by their ends to the fibers, in which case they appear rod-shaped. During anaphase the chromosome do not split preparatory to the homoeotypic mitosis. They pass entire to the poles where they form a compact little cluster, one chromosome usually occupying the center of the group with the other six lying around it (fig. 32). About this little group there develops a small vacuole-like region, the outer boundary of which becomes the nuclear membrane. At the same time a few little threads are here and there thrown across from one chromosome to another, forming slight connections.

Interkinesis

The daughter nuclei, so constituted, grow rapidly. The individual chromosomes are thus enabled to separate more and more, and tend to take a parietal position. As the nucleus increases in size they become more irregular in outline, but always remain perfectly distinct and separate, being attached to each other only in the most meager way by a few scant threads (fig. 33). Nucleoli also begin to make their appearance at this time, appearing at first as minute, faintly staining globules, which gradually grow in size, never becoming large, however, or appearing to exercise any important function (figs. 33, 34). There are usually several of them in a nucleus, and they develop in contact with the chromosomes or with the chromatin threads. The fact that several of them appear synchronously in various parts of the nucleus, and that they seem to have such an intimate connection with the chromosomes, suggests that they arise *de novo*, and are not descended from previously existing bodies such as the endonucleolus.

It is not until late interkinesis that the irregularity in the shape of the chromosomes begins to give place to a definiteness of form. Shortly before the disappearance of the nuclear membrane preparatory to the homoeotypic mitosis, it becomes clear that the chromosomes have split, and that the halves are swinging around in such a way that they cross. Oftentimes they bend over one another in a very interesting way (fig. 34), at other times they remain straight. In either event, the general appearance is that of a Maltese cross.

The Homoeotypic Division

The spindle for the homoeotypic mitosis begins to develop shortly before the nuclear membrane breaks down. Here again a very prominent multipolar spindle is formed, which later becomes bipolar. The chromosomes, which lie scattered in the cytoplasm after the disappearance of the nuclear membrane, become very short and are clearly paired. They are brought to the equatorial region (fig. 35) and are separated, and seven chromosomes pass to each pole in an entirely regular way. The two figures lie at opposite sides of the cell, with their axes in a majority of cases in the same plane, though this position is not constant (fig. 35).

The granddaughter nuclei are constituted in much the same manner as were the daughter nuclei. The seven chromosomes lie at first very close together, and become surrounded by a vacuole whose outer boundary becomes the nuclear membrane. The nuclei grow rapidly and pass quickly into a resting state in which all trace of the individual chromosomes is lost. The process of expansion of the chromosomes is very clear and can easily be followed (figs. 36–38). Each chromosome begins to lengthen out and becomes a thread, which loses in diameter as it gains in length (fig. 36). Each of these threads may attain a length equal to, or greater than, the diameter of the nucleus. As each chromosome lengthens, it can be seen that very delicate strands begin to extend out from it in every direction, and these threads, coming from the various chromosomes, meet and fuse, forming a very delicate reticulum. We have then at this stage seven prominent threads, joined together by a loose network of very delicate ones (fig. 37). The contents of the original threads then pass out in varying degree into the more delicate strands, so that in time it becomes impossible to tell where the original chromosomes lay (fig. 38). After the material is thus rather thoroughly distributed, and the nucleus has reached the resting condition, it is seen to possess a loose reticulum, dotted over with chromatin particles of various sizes, and also one to several nucleoli, which have arisen as in the daughter nuclei. It is especially to be noted that there is no indication of a splitting of the chromosomes during this period. The process seems to be one purely and simply of expansion—increase in length of the original bodies, formation of branch threads by an outflowing of linin material, and distribution of the chromatin contents along them. Whether or not this telophase may be considered as throwing any light upon the telophase problem brought up by Digby (1919) may of course be open to question.

The four nuclei come to lie equidistant from one another, so that when the new walls are formed the resultant spores are arranged in tetrahedral fashion. Between them, more or less prominent sets of spindle fibers often appear. Sometimes these are very marked, at other times there is hardly a trace of them, so that when present they are probably functionless. The walls separating the spores are not formed until after the nuclei have entered fully upon the resting stage. They are all formed at one time, appearing at first as delicate cleavage planes, which later become thicker and more prominent. If cleavage begins at the surface of the cell, it passes to the center with great rapidity. I am inclined to believe that it may be brought about almost simultaneously everywhere in the planes where the walls are to form. There is no evidence that the walls develop centrifugally, by the formation of cell plates between the nuclei. No cell plates were observed, even in cases in which the spindles were most prominent, and there can be but little doubt that the division is the result of very rapid furrowing.

DISCUSSION

The results of this investigation are of interest from the standpoint of nuclear cytology, and also because of their bearing upon problems in Oenothera genetics. We will first take up the cytological questions involved, and follow these with a brief consideration of the genetical significance of the results.

Cytological

Telosynapsis vs. Parasynapsis. One of the first questions that naturally arises during the study of maturation divisions concerns the arrangement of the chromosomes and the nature of the spireme thread. The evidence in this case seems to lead one to a telosynaptic interpretation. During the early heterotypic prophase, there is little to lead one to suppose that the chromosomes are undergoing synapsis. For the most part, the threads are unpaired. The occasional bits of parallelism to be seen at this stage are about as much in evidence as one would ordinarily expect in the transformation of a reticulum into a spireme. They are not sufficiently numerous or sufficiently extended to warrant one in considering them evidences, either for the synapsis of whole chromosomes, or for the fusion of the halves of split chromosomes. Although I have not been able to study the somatic telophases antecedent to the heterotypic prophase, the results which Sharp (1913, 1920) has obtained from the study of Vicia and Tradescantia will probably hold perfectly well in *Oenothera franciscana* also. He has found that the alveolation of the chromosomes in telophase is too irregular to be called a splitting, and that in prophase the chromosomes are fully formed by the reverse process of condensation before a split occurs. I have come strongly to the conclusion that the spireme in the heterotypic prophase of this plant is formed by an irregular process of condensation, rather than by the approximation of two distinct threads. This conclusion not only conflicts with the idea of an early split in the chromosomes of this plant, preparatory to the homoeotypic mitosis, but also with that of the possibility of synapsis, or side-by-side approximation of whole chromosomes, taking place during this period.

During synizesis, the process can be seen to be a continuation of that described above. Little indication of parallelism of any kind can be found, and what little bit there is, is only what one might expect from chance, or from the unequal condensation of the various threads of the system. When the spireme is finally formed, all the evidence seems to support the theory that it is univalent in nature. From late synizesis to the end of the second contraction stage, it remains uniformly and evenly single. As it begins to condense and shorten, so that the long, tangled loops into which it is thrown stand out more and more clearly, the single nature becomes all the more noticeable, for it is at this period that the split, in many plants and animals, becomes especially evident, and the separated threads twist around one another forming the characteristic "strepsinema" condition. In this plant, however, there is no true strepsinema, or twisting of threads, for there is no split in the spireme. The split in preparation for the homoeotypic mitosis does not appear in this plant, except rarely, until the middle or end of interkinesis.

As the spireme becomes more and more massed in the center of the nucleus during second contraction, the loops become very short and thick, and it can be seen that the chromosomes forming the loops are attached end to end. These loops can be traced throughout the whole of the second contraction period, and it seems quite certain that the ring-shaped bivalent chromosomes which emerge from the knot are the loops which entered it. Each bivalent seems to have come, therefore, from an unsplit section of the spireme. It is true that toward the end of the second contraction period I have found a very few cases in which there seemed to be some evidence of a split. These instances were so rare, however, in comparison with the cases in which the thread seemed perfectly whole, that the only interpretation possible is that they represent a premature split (premature for this plant, though usual in most if the telosynaptic view is correct) in preparation for the homoeotypic division.

Diakinesis also presents strong evidence for the telosynaptic arrangement of chromosomes in the constant and uniform linking together of bivalent chromosomes, a phenomenon which can hardly be explained on a parasynaptic basis. The most reasonable explanation seems to be that the bivalent chromosomes represent sections of the spireme which occupied such a position in the nucleus that, when the whole system became condensed, the chromosomes found themselves linked in this very definite way. It is not likely that a chromosome could pass between two members of a bivalent pair in the way seen in this plant, if those two members originally lay side by side in the same spireme. The univalent chromosomes making up a bivalent, if attached at all during the open spireme, must have been attached end to end.

On the whole, therefore, the singular absence of parallelism at all stages, the clear relationship established between the second contraction loops and the bivalent chromosomes, and the regular linking of the latter during diakinesis, seem to make it quite certain that we are dealing here with chromosomes which are attached end to end in a univalent spireme.

The Position of the Chromosomes. The regularity with which the bivalent chromosomes are linked together in diakinesis raises an interesting question concerning the relative positions of the chromosomes in the nucleus. The fact that linking seems to conform to a definite scheme suggests that during the preceding spireme stage the individual chromosomes occupied the same relative position in all nuclei, being attached to each other at the same places and in the same way. How far back this condition existed is probably difficult, if not impossible, to determine in a plant where the individuality of the chromosomes is lost sight of during resting stages; but at least the interesting possibility is suggested that such a definite arrangement might be present in all nuclei. Whatever may be the situation in the somatic cells in general, it is quite probable that the chromosomes are arranged during the last archesporial mitosis according to a definite plan, so that they come to occupy the same relative positions in the subsequent prophase stages.

The Nucleolus. There is always at least one nucleolus in the nucleus of every pollen mother cell in *Oe. franciscana.* That its function seems to be at least partially that of storage is indicated by the appearance presented during late synizesis, when the threads surrounding and attached to the nucleolus become greatly swollen, as though gorged with material suddenly poured into them. At the same time, the nucleolus begins to lose its staining qualities and to look empty. The material which it stores stains intensely with the heamatoxylin, and would seem, therefore, to be a form of chromatin. The nucleolus itself, when divested of the chromatic contents, does not take the stain and would therefore seem to be composed of a substance perhaps closely related to, or identical with, linin. At different times it presents a somewhat different appearance. Before discharging its contents, it contains at times a crystalloid body. In other cases one or more vacuoles are present in the interior. After it has been emptied of its chromatin content, it very generally shows within a black-staining, spherical little body, the endonucleolus. This seems at times to be connected directly with the spireme by a prominent thread, but usually it lies freely in the nucleolus. It outlasts the nucleolus ordinarily, being occasionally seen at metaphase, but disappears in time without having undergone division, and seems therefore to have no part to play in the development of the nucleoli in the daughter nuclei. The fact that during interkinesis several new nucleoli can be seen to develop simultaneously in contact with the chromosomes, and probably from them, makes it seem probable that the nucleoli arise *de novo*, and not from preëxisting ones. The nucleolus seems, therefore, to be a body which stores material of some sort, probably chromatin, and which is formed anew in each nucleus.

Genetical

It has been very clearly pointed out by Davis (1915) that the chief source of doubt concerning the validity of many of the interpretations based on breeding experiments in the genus Oenothera lies in the ever-recurring suspicion that the species under consideration are not genetically pure. He has shown that the mere fact of breeding true is not a sufficient test of purity, in view of the large percentage of pollen and seed sterility often encountered in these plants, which phenomena introduce an element of doubt, in that they allow of the possibility of the presence of more than one type of gamete, only one of which may survive to function, or, if they all function, to produce successful progeny. The element of doubt thus introduced into the study of even those species which seem for the most part to breed true tends to cast a shadow of suspicion upon the conclusions based upon this work. It becomes, therefore, a matter of great importance to develop methods by which the purity of a species may be satisfactorily tested, and to find species whose purity is beyond question.

The main barrier in the way of determining whether the members of the *Lamarckiana* group are pure or impure is the high percentage of sterility there found. Until the reasons for this sterility are determined, it is impossible to tell whether "mutants" thrown by them are indeed mutants, or whether they are Mendelian segregates. Meanwhile, the abundant sterility is itself a possible indication of hybridity. There are, however, a few species of Oenothera in which the percentage of sterility is lower than in most of the *Lamarckiana* group, and which at the same time breed quite true. At least one of these species, however, *Oe. biennis* L., is not entirely above suspicion. While it breeds remarkably true in the main, it has nevertheless been shown by Stomps (1914) to throw a small percentage of "mutants." Furthermore, it shows over 50 percent pollen sterility. The other species, however, which may prove to be the long-looked-for pure species, are *Oe. grandiflora* and *Oe. franciscana*. Both of these are extremely stable, and at the same time show a very small percentage of both pollen and seed sterility.

The latter species has proved absolutely stable thus far, as is shown by table 1, published herewith through the kindness of Dr. Davis.

TABLE 1. *History of Oenothera franciscana B.*

Year	Culture	Source of Culture (Culture and Plant)	Description of Culture
1913.......	13.21	Bartlett 10_6–12_6	52 plants, uniform
1914.......	14.21	13.21*a, c*	18 plants, "
1915.......	15.21	14.21*a*	16 plants, "
1916.......	16.21	15.21	9 plants, "
1917.......	17.21	16.21*a*	12 plants, "
1918.......	——	——	No culture
1919.......	19.21	17.21, III–1	15 plants, uniform
1920.......	20.21	19.21, III–2	25 plants, "
1921.......	21.21	20.21, 5	1,352 mature plants 21 remaining as rosettes 1,373 uniform and typical
Total.....			1,520 uniform and typical

It will be seen from this table that during the eight years in which this strain has been carried along, not a single mutation or aberrant form has been discovered. There is very little pollen sterility, and the seed tests made by Dr. Davis (table 2) show that the percentage of seed sterility is also very small.

TABLE 2. *Seed Fertility in Oenothera franciscana B.*

Year	Culture	Procedure	Result of Test	Percentage of Germination
1915....	15.21	Contents of two capsules, seeds germinated in earth	442 seedlings 280 seed-like structures 722	61%
1919....	19.21	Contents of one capsule, soaked 24 hours. Subjected to alternate exhaust and pressure (50 lbs.) twice in 24 hours	398 seedlings 43 seed-like structures 441	90.2%
1921....	21.21	Contents of three capsules, soaked 24 hours. Subjected to alternate exhaust and pressure (30 lbs.) 7 times in 24 hours	1,425 seedlings 209 seed-like structures 1,634	87.3%

All the indications so far, then, point to *Oe. grandiflora* and *Oe. franciscana*, and especially to the latter species, as likely to prove genetically pure. In view of this fact, it becomes a matter of extreme interest to investigate these plants cytologically and to compare them from this standpoint also with the members of the *Lamarckiana* group. The maturation divisions in the pollen mother cells of *Oe. grandiflora* have been studied by Davis (1909). He made the interesting discovery that in this species the seeming incompatibility between homologous chromosomes, which is so characteristic of the plants of the *Lamarckiana* series, and which seems in their case to point strongly to a hybrid nature, is lacking. The homologous chromosomes pair with entire regularity, and are carried to the equatorial plate and separated in a perfectly typical manner. In view of this suggestive study, it becomes of interest also to make a cytological study of *Oe. franciscana*, which is even more strikingly uniform in its behavior than *Oe. grandiflora*. The results of this study have been very interesting as indicating an even more striking regularity of chromosome behavior during the maturation divisions than that found in *Oe. grandiflora*. Not only do the chromosomes regularly pair with one another, but they show a most interesting uniformity of position, resulting in the linking of bivalent chromosomes in a seemingly constant manner; and, most striking of all, a definite morphological peculiarity, in the nature of a closed circle of four univalent chromosomes, is found to be always present during diakinesis, a feature which is most readily observable, and whose constancy is a clear reflection of the uniformity which characterizes the activity of the chromosomes as a whole at this time.

It seems to be a fact, therefore, that the failure of homologous chromosomes to pair at diakinesis, and the tendency for the chromosomes to come to the equatorial plate in an irregular fashion, are not characteristic of the genus Oenothera as a whole, but only of those species which have been found to be unstable. In the stable species, homologous chromosomes seem to have a strong affinity for one another during diakinesis. In the unstable species, they seem to have little or no affinity for one another, and the most obvious inference is that this incompatibility is due to the hybrid nature of the species. Such an inference may not be correct, however, as it may turn out, when a careful cytological and physiological investigation is made of pollen and ovule development in the more unstable species, that there is but one type of gamete present for each sex. If such be the case, then the irregularity of chromosome behavior is to be considered rather a morphological expression of the peculiar condition, physiological or morphological, which results in the tendency to mutate.

However this may be, the correlation between hereditary instability and the irregular behavior of chromosomes on the one hand, and between stability and the regular, uniform behavior of chromosomes on the other, is rather striking. Not only does it emphasize the probable purity of the stable species, but by contrast it should make one all the more cautious about considering the unstable ones as genetically pure.

In conclusion, I wish to thank Dr. B. M. Davis for his great kindness in allowing me to make use of material growing in his gardens, in permitting me to include in this paper some of his unpublished data, as well as for the interest which he has manifested in the study as it has progressed.

SUMMARY

1. The nucleus passes through a definite resting period of considerable length, previous to entering upon the heterotypic prophase. During this period all signs of the individual chromosomes are lost. The threads are unpaired.

2. The approach of prophase is indicated by occasional, though not very striking, parallelisms in the network, due to the contraction of threads here and there. There is no general pairing of threads such as has been described by many workers on other forms.

3. Prophase is ushered in by a general contraction of the reticulum, which soon causes the threads to be torn away from most of the periphery and to shrink into a tight knot—the synizetic knot.

4. During synizesis, many of the threads disappear and their contents pass into other threads, which thus become more and more prominent, and finally emerge from the knot as the spireme. There is very little parallelism of threads during synizesis, and what little there is seems to be due merely to the way in which the threads condense. There is no reason for believing that it represents the fusion of the separated halves of a univalent spireme, or the synapsis of two univalent spiremes to form a bivalent one.

5. There is a well marked "open-spireme" stage, during which the threads are fairly uniform in diameter and display no evidence of doubleness. There is no strepsinema condition, the nucleus passing into the second contraction by the gradual accumulation of material in the center of the nucleus, and by the contraction of the spireme thread into smaller and smaller loops radiating from the center, the threads composing these loops being single in nature.

6. The loops can be traced uninterruptedly through the second contraction, and undoubtedly form the bivalent chromosomes which emerge at the end of this period. The univalent chromosomes are arranged end to end, or telosynaptically, in these loops, the places where they join being marked by constrictions.

7. The bivalent chromosomes emerge in a definite way from the second contraction, and are arranged in such a manner that they form one closed circle, consisting of four univalent chromosomes, and five rings composed each of two chromosomes, all of which are linked together at first according to a definite scheme. Homologous chromosomes are perfectly compatible.

8. During diakinesis, these rings separate to a greater or less extent from one another. Each individual ring, however, including the circle of four chromosomes, remains intact.

9. The rings are drawn to the equatorial plate at metaphase. The circle of four chromosomes breaks into two pairs. The rings are arranged in a flat plate, and the univalent chromosomes are separated and carried to opposite poles without the least sign of irregularity.

10. The longitudinal split in the daughter chromosomes preparatory to the homoeotypic division does not appear until late interkinesis. During interkinesis, the individual chromosomes remain clearly visible, being attached in only the most meager way.

11. The homoeotypic division is in no respect unusual and is followed by the simultaneous cutting in of walls to form the four spores of the tetrad.

12. The nucleus of each spore passes into a resting condition in which all trace of the individual chromosomes is lost. This process is easily followed, and it is clear that no splitting of the chromosomes into two threads takes place, as has been described in the telophase of some plants.

13. The chromosomes in this plant are arranged telosynaptically, and form a univalent spireme during prophase of the heterotypic mitosis.

14. The reduction processes in *Oenothera franciscana*, which is genetically a very stable species, are extremely regular and typical, in striking contrast with what is found to be the case in the less stable species of the genus, but in agreement with the condition which has been found in one of the other stable species, *Oe. grandiflora.*

GOUCHER COLLEGE

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, using a Spencer high-powered binocular microscope, with Spencer 1.8 oil-immersion objective and 10X eye-piece. They were enlarged by the use of a pantograph, and reduced one fourth in reproduction. Magnification 2,280 diameters, except figure 35, which is at a magnification of 1,140.

PLATE XXV

FIGS. 1, 2. Resting stage before heterotypic prophase.

FIG. 3. Parallelism, due to contraction of threads here and there, indicating approaching prophase.

FIG. 4. Beginning of prophase.

FIG. 5. Network is beginning to contract to form synizetic knot.

FIGS. 6, 7. Tangential sections during early synizesis, showing disappearance of some threads and augmentation of others.

FIGS. 8, 9. Later synizesis. The threads becoming more uniform and thicker.

FIG. 10. Spireme well formed. Threads near the nucleolus gorged with material.

FIGS. 11, 12. End of synizesis. Thread single and uniform except near the nucleolus, often beaded. Endonucleolus present.

PLATE XXVI

FIG. 13. Same as last two figures. Nucleolus returning to the center.

FIG. 14. Open spireme stage. Notice connection between the endonucleolus and the spireme. (See also fig. 16.)

FIG. 15. Beginning of second contraction. Central portion of reticulum begins to thicken. Peripheral portion thrown into radiating loops.

FIGS. 16–18. Later stages in condensation, the central region larger, the peripheral threads thicker and shorter. Note position of nucleolus in figure 18.

FIG. 19. Further stage in contraction. Note chromosome forming the distal end of a loop. The sides of two of the loops seem to have partially fused together. This stage is frequently seen.

FIG. 20. Loops, or bivalent chromosomes, radiating from the central region.

FIG. 21. The climax of contraction. Loops still plainly seen, however.

FIG. 22. Second contraction knot begins to loosen. Two rings at one end and one at the other.

FIG. 23. Slightly later stage. One loop at the top, two rings at the bottom of the figure. Usually at about this stage one or more bivalent chromosomes separate entirely from the rest. Note endonucleolus.

FIG. 24. Circle of four chromosomes, to which are linked two two-linked chains, each link being a pair of chromosomes.

PLATE XXVII

FIG. 25. Circle of four, with one two-linked chain attached. Three rings separated.

FIG. 26. Circle of four, with three rings attached.

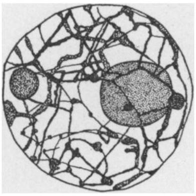
FIG. 27. Circle of four, with one two-linked chain, and one single ring attached.

FIG. 28. Circle of four, and five separate rings. Mid-diakinesis. Nucleolus melting.

FIG. 29. Multipolar spindle. The circle of four unbroken. Two rings still linked to it. Endonucleolus still present.

FIG. 30. Metaphase of the heterotypic mitosis. Note regularity of arrangement.

FIG. 31. Anaphase of the heterotypic mitosis.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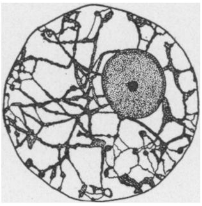
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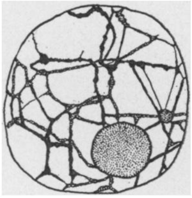
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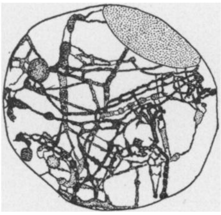
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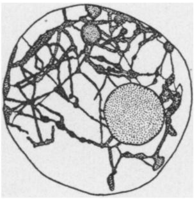
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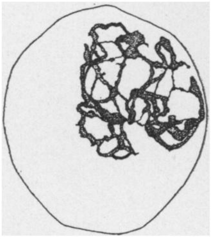
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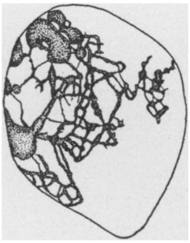
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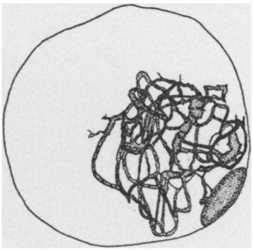
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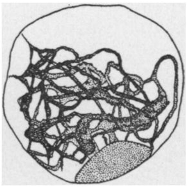
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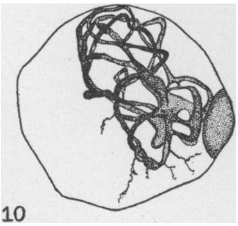
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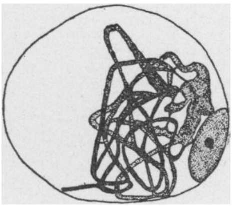
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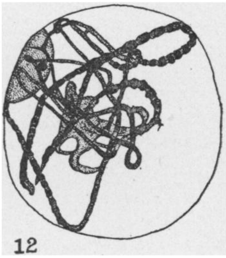
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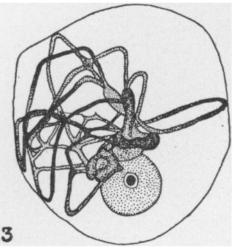
Cleland: Oenothera franciscana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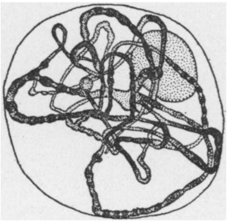
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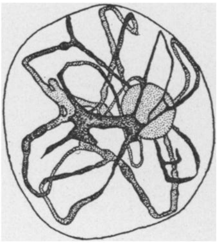
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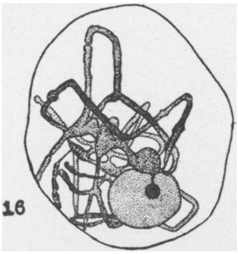
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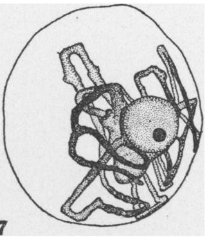
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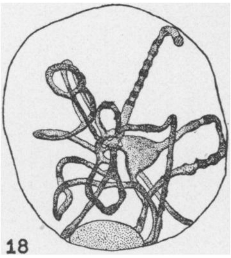
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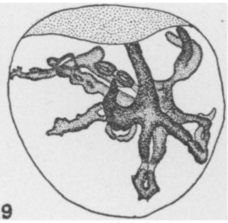
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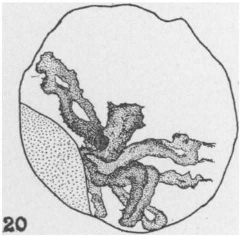
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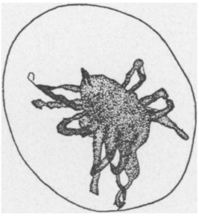
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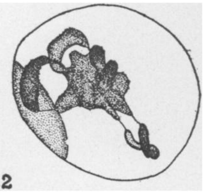
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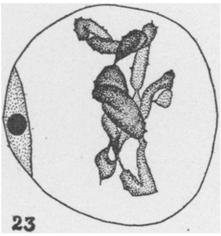
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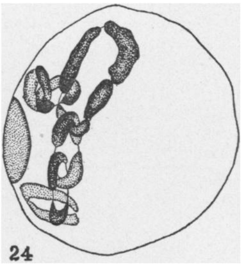
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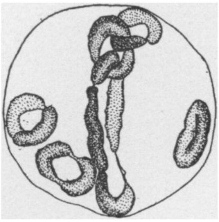
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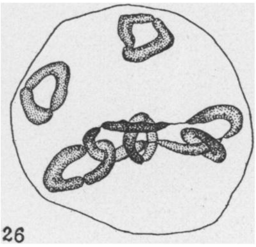
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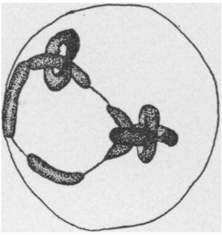
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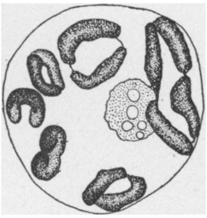
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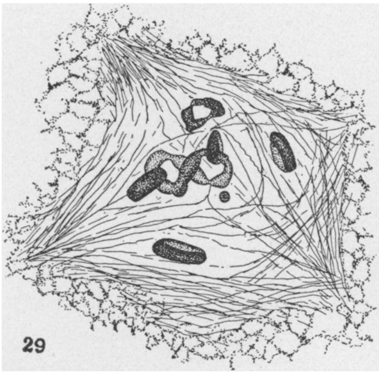
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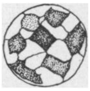
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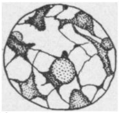
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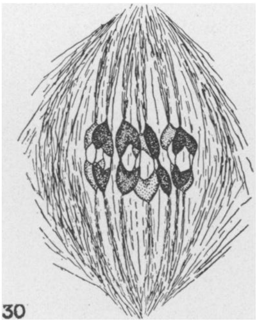
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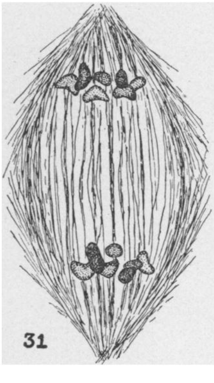
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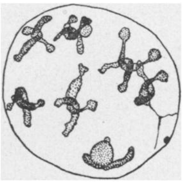
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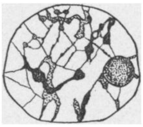
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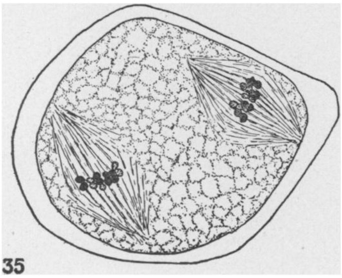
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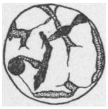
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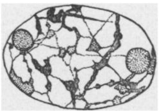
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FIG. 32. Early interkinesis.

FIG. 33. Later. Chromosomes distinct, but to all appearances as yet unsplit.

FIG. 34. Late interkinesis. Chromosomes have split, and halves have bent around each other.

FIG. 35. Metaphase of the homoeotypic mitosis. × 1,140.

FIG. 36. Granddaughter nucleus, soon after formation. Chromosomes are beginning to lengthen and to send out delicate projections, which later fuse to form a network.

FIG. 37. Chromosomes are rapidly losing their identity, as their contents pass out into the projecting threads.

FIG. 38. Resting stage in a granddaughter nucleus. Chromosomes are entirely obscured.